

REPORT DOCUMENTATION PAGE		<i>Form Approved</i> OMB No. 0704-0188	
The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.			
1. REPORT DATE (DD-MM-YYYY) 28-11-2014		2. REPORT TYPE Final	
		3. DATES COVERED (From - To) 28 Feb 13 – 19 Sept 14	
4. TITLE AND SUBTITLE Modular organization of dynamic camouflage body patterning in cuttlefish		5a. CONTRACT NUMBER FA2386-13-1-4052	
		5b. GRANT NUMBER Grant AOARD-134052	
		5c. PROGRAM ELEMENT NUMBER 61102F	
6. AUTHOR(S) Dr Chuan-Chin Chiao		5d. PROJECT NUMBER	
		5e. TASK NUMBER	
		5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) National Tsing Hua University 101, Sec 2, Kuang-Fu Rd., Hsinchu 30013 Taiwan		8. PERFORMING ORGANIZATION REPORT NUMBER N/A	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) AOARD UNIT 45002 APO AP 96338-5002		10. SPONSOR/MONITOR'S ACRONYM(S) AFRL/AFOSR/IOA(AOARD)	
		11. SPONSOR/MONITOR'S REPORT NUMBER(S) AOARD-134052	
12. DISTRIBUTION/AVAILABILITY STATEMENT Distribution A: Approved for public release. Distribution is unlimited			
13. SUPPLEMENTARY NOTES			
14. ABSTRACT Cephalopods (octopus, squid, and cuttlefish) can camouflage themselves against almost any background. Yet their ability to quickly alter their body patterns on different visual backgrounds has posed a research challenge: how they are able to pick the correct pattern amongst their repertoire. In cuttlefish, these studies and others have demonstrated that their ability to change skin coloration appropriately requires a visual system that can rapidly assess complex visual scenes and produce the motor output - the neutrally controlled body patterns - that achieves camouflage. The goal of this project was to study the neural basis of camouflage in cuttlefish, <i>Sepia pharaonis</i> , by combining behavioral assay with neurophysiological stimulation. In addition the oval squid <i>Sepioteuthis lessoniana</i> was used to make a parallel investigation and to broaden the scope of modular organization of body patterning. By applying electrical stimulation to systematically characterize the motor fields of command units in the optic lobe that control distinct body patterns in cuttlefish and oval squids, it was found that (1) the responsive areas are positively correlated with increasing voltages and depths of the electrode in the medulla of the optic lobe, and (2) the island-like clumps with a hierarchical organization in the medulla of the optic lobe are responsible for generating different body patterns. Although the original aim of using the dynamically changing visual background to study the spatiotemporal expression of body patterns was not successful, we discovered that sudden changing visual background can elicit threatening behaviors and may provide a new means of studying dynamic body patterning in the future. In conclusion, this project demonstrates that adaptive body coloration in cephalopods is based on the hierarchical principle of modular organization in the higher command center of the brain.			
15. SUBJECT TERMS Biochemistry			

Report Documentation Page		Form Approved OMB No. 0704-0188
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.		
1. REPORT DATE 28 NOV 2014	2. REPORT TYPE Final	3. DATES COVERED 28-02-2013 to 19-09-2014
4. TITLE AND SUBTITLE Modular organization of dynamic camouflage body patterning in cuttlefish		5a. CONTRACT NUMBER FA2386-13-1-4052
		5b. GRANT NUMBER
		5c. PROGRAM ELEMENT NUMBER 61102F
6. AUTHOR(S) Chuan-Chin Chiao		5d. PROJECT NUMBER
		5e. TASK NUMBER
		5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) National Tsing Hua University,101, Sec 2, Kuang-Fu Rd,Hsinchu 30013,Taiwan,TW,30013		8. PERFORMING ORGANIZATION REPORT NUMBER N/A
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) AOARD, UNIT 45002, APO, AP, 96338-5002		10. SPONSOR/MONITOR'S ACRONYM(S) AFRL/AFOSR/IOA(AOARD)
		11. SPONSOR/MONITOR'S REPORT NUMBER(S) AOARD-134052
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited		
13. SUPPLEMENTARY NOTES		
14. ABSTRACT Cephalopods (octopus, squid, and cuttlefish) can camouflage themselves against almost any background. Yet their ability to quickly alter their body patterns on different visual backgrounds has posed a research challenge: how they are able to pick the correct pattern amongst their repertoire. In cuttlefish, these studies and others have demonstrated that their ability to change skin coloration appropriately requires a visual system that can rapidly assess complex visual scenes and produce the motor output - the neutrally controlled body patterns - that achieves camouflage. The goal of this project was to study the neural basis of camouflage in cuttlefish, Sepia pharaonis, by combining behavioral assay with neurophysiological stimulation. In addition the oval squid Sepioteuthis lessoniana was used to make a parallel investigation and to broaden the scope of modular organization of body patterning. By applying electrical stimulation to systematically characterize the motor fields of command units in the optic lobe that control distinct body patterns in cuttlefish and oval squids, it was found that (1) the responsive areas are positively correlated with increasing voltages and depths of the electrode in the medulla of the optic lobe, and (2) the island-like clumps with a hierarchical organization in the medulla of the optic lobe are responsible for generating different body patterns. Although the original aim of using the dynamically changing visual background to study the spatiotemporal expression of body patterns was not successful, we discovered that sudden changing visual background can elicit threatening behaviors and may provide a new means of studying dynamic body patterning in the future. In conclusion, this project demonstrates that adaptive body coloration in cephalopods is based on the hierarchical principle of modular organization in the higher command center of the brain.		

15. SUBJECT TERMS Biochemistry					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified	Same as Report (SAR)	17	

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Kristopher, Ahlers, Lt Col, USAF, Ph.D.
U	U	U	SAR	17	19b. TELEPHONE NUMBER <i>(Include area code)</i> +81-3-5410-4409

Standard Form 298 (Rev. 8/98)
Prescribed by ANSI Std. Z39.18

Modular organization of dynamic camouflage body patterning in cuttlefish

A final report for AFOSR/AOARD

FA2386-13-1-4052

20 Mar 2013 – 19 Sep 2014

Principal investigator: Chuan-Chin Chiao, PhD

Program manager: Lt. Col Jermont Chen, jermont.chen@us.af.mil

Lt. Col Kris Ahlers, kristopher.ahlers@us.af.mil

Primary affiliation

Professor, Department of Life Science

Director, Institute of Systems Neuroscience

National Tsing Hua University

Hsinchu, 30013

TAIWAN

Tel: 886-3-5742464

Fax: 886-3-5715934

Email: ccchiao@life.nthu.edu.tw

Secondary affiliation

Adjunct Senior Scientist, Program in Sensory Physiology & Behavior

Marine Biological Laboratory

Woods Hole, MA 02543

USA

Summary

Cephalopods (octopus, squid, and cuttlefish) can camouflage themselves against almost any background. Yet their ability to quickly alter their body patterns on different visual backgrounds poses a vexing challenge: how to pick the correct pattern amongst their repertoire. In cuttlefish, our studies and others have demonstrated that their ability to change skin coloration appropriately requires a visual system that can rapidly assess complex visual scenes and produce the motor output - the neutrally controlled body patterns - that achieves camouflage. To gain further insight into neural processing of dynamic body patterning, the goal of this project was to study the neural basis of camouflage in cuttlefish, *Sepia pharaonis*, by combining behavioral assay with neurophysiological stimulation. In addition, we also used a different cephalopod species, the oval squid *Sepioteuthis lessoniana*, to make a parallel investigation and to broaden the scope of modular organization of body patterning. By applying electrical stimulation to systematically characterize the motor fields of command units in the optic lobe that control distinct body patterns in cuttlefish and oval squids, we found that (1) the responsive areas are positively correlated with increasing voltages and depths of the electrode in the medulla of the optic lobe, and (2) the island-like clumps with a hierarchical organization in the medulla of the optic lobe are responsible for generating different body patterns. Although the original aim of using the dynamically changing visual background to study the spatiotemporal expression of body patterns was not successful, we discovered that sudden changing visual background can elicit threatening behaviors and may provide a new means of studying dynamic body patterning in the future. In conclusion, this project demonstrates that adaptive body coloration in cephalopods is based on the hierarchical principle of modular organization in the higher command center of the brain.

Introduction

Modern cephalopods (octopus, squid, and cuttlefish) form a group called Coleoidea in the phylum Mollusca. They are soft-bodied invertebrates and appeared in the ocean roughly at the same time as teleost fish some 200 million years ago. Unlike its sister group Nautiloidea (*Nautilus*), which has a rigid outer shell for protection, coleoids have at most an internal bone that is used for buoyancy control (e.g., cuttlefish). Some species have lost their bone altogether (e.g., octopus), while in some it has been replaced by a thin cartilaginous support structure (e.g., squid). These modern cephalopods have keen vision and elaborate camouflage body patterning, and they have evolved into highly effective hunters in the sea. However, these soft-bodied creatures are also the highly nutritious prey of many marine predators (fish, marine mammals, and even birds), thus the selection pressure on them is enormous throughout the evolution. It is likely that strong competition between cephalopods and fish led coleoids to evolve complex brains and dynamic camouflage body patterns (Hanlon and Messenger, 1996).

Camouflage versatility is probably no better developed in the animal kingdom than in the coleoid cephalopods. These marine animals possess an elaborate sensorimotor system that controls body pattern expression for camouflage and communication (Messenger, 2001). Most animals have a fixed or slowly changing camouflage pattern, but cephalopods have evolved a different defense tactic: they use their keen vision and sophisticated skin – with direct neural control for rapid change and fine-tuned optical diversity – to rapidly adapt their body pattern for appropriate camouflage against a staggering array of visual backgrounds, including colorful coral reefs, temperate rock reefs, kelp forests, sand or mud plains, seagrass beds, and others, apparently without color vision (Marshall and Messenger, 1996; Mäthger et al., 2006; Hanlon et al., 2011; Chiao and Hanlon, 2013).

The eye as a sensor of diverse visual backgrounds

Testing the visual cues that drive the adjustment of body patterning is possible with cephalopods. Cuttlefish (*Sepia* spp.) are particularly suited for this task because they are well adapted to laboratory environments and they are, like many shallow-water benthic (bottom-dwelling) cephalopods, behaviorally driven to camouflage themselves on almost any background. Thus, both natural and artificial backgrounds can be presented to cuttlefish in order to observe their camouflage responses.

How many camouflage patterns do cephalopods have? Our extensive field and laboratory observations of cuttlefish camouflage (Hanlon and Messenger, 1988), surprisingly and counter-intuitively, revealed only three basic patterning templates among thousands of cuttlefish images: uniform, mottle, and disruptive. Furthermore, camouflage patterns on more than 20 cephalopod species can be grouped into these three categories as well (Hanlon and Messenger, 1996). Of course, there is variation within each broad pattern class. Such classification into three named categories is partly descriptive, but quantitative methods support that these pattern categories are based on statistical properties of animal skin patterning (Hanlon et al., 2009).

Which properties of the background determine whether a cuttlefish will produce a uniform, mottle, or disruptive pattern? This issue has received much attention over the past decade (Hanlon et al., 2011). Three of the most important factors are: (1) the spatial frequency content of the background (Chiao et al., 2005; Kelman et al., 2007), (2) the contrast of the background (Barbosa et al., 2008; Chiao et al., 2009, 2010; Zylinski et al., 2009a), and (3) whether or not the background contains any light elements of roughly the same size as the cuttlefish's "white square" (a rectangular skin patch on the dorsal mantle; Chiao and Hanlon, 2001ab; Barbosa et al., 2007). In addition, increasing substrate luminance tends to attenuate the production of disruptive patterns (Chiao et al., 2007). The edge of objects and visual depth also provide salient cues in evoking disruptive patterns (Chiao et al., 2005; Kelman et al., 2008; Zylinski et al., 2009b).

Despite extensive studies in visual perceptual principles of camouflage body patterning in cuttlefish (i.e., the correlations between sensory input and motor output), little is known about the neural processing in the brain that controls body pattern generation. Understanding the neural principle responsible for coordinating spatiotemporal expression of chromatophores in the skin to generate dynamic camouflage body patterns will provide insights into mechanisms of pattern formation and pattern expression in diverse biological and non-biological systems.

Changeable skin that enables optical and physical malleability

The skin of cephalopods is a marvelous example of rapid, highly coordinated optical malleability. Pigmentary and structural coloration are combined in many ways to achieve vastly different appearances, both from close-up and distant viewing. Pigmented chromatophore organs (yellow, red, or brown in most cephalopods) are actively controlled neurophysiologically from the brain. Cell bodies in the chromatophore lobes in the suboesophageal brain travel without synapse to radial muscles that implement expansion/retraction of the pigment sacs of chromatophores with maximal speed (Messenger, 2001). Subjacent to the chromatophores are iridophores, and below them are leucophores, which diffuse ambient light equally in all directions and act as a base layer upon which dark patterning is layered. Iridophores are directional structural reflectors, some of which are passive cells and some of which are controlled actively (Wardill et al., 2012). They tend to reflect the short wavelength colors that complement those of the longer wavelengths of the chromatophores (Mäthger et al., 2009).

Neural control of dynamic body patterning

Dynamic body patterning in cephalopods is controlled by a set of lobes in the brain organized hierarchically. At the highest level, the optic lobes select specific motor commands (i.e., body patterns), largely based on visual information; at the lowest level, motor neurons in the chromatophore lobes execute these commands and produce the patterning seen in the skin. Although the chromatophore system has been extensively studied in past decades (review in Messenger, 2001), the neural basis and organizational principle of these motor commands in the optic lobe is largely unknown. By studying chromatomotor fields, Packard (1982, 1995) was the first to classify groups of chromatophores in the skin into “morphological” and “physiological” units. Since a single chromatophore receives multiple innervations, a particular chromatophore (or group of chromatophores) can participate in different body patterns. Based on these findings, it was suggested that these neurally activating units in the skin constitute diverse body patterns in cephalopods. In a subsequent study, by carefully observing cuttlefish body patterns in the field and laboratory, Hanlon and Messenger (1988) deduced 34 chromatic components that are responsible for various cuttlefish skin colorations.

However, it is not known if these distinct chromatic components are controlled independently in the brain or if they are equivalent to the physiological units described by Packard. In a separate study, by applying multivariate analysis on an ensemble of cuttlefish images, such as independent component analysis (ICA), Anderson et al. (2003) showed that the diverse body patterns seen in cuttlefish can be created by combining a small number of distinct pattern modules or templates, which suggests a modular organization of body patterning. Despite the success of ICA, the question of whether these independent pattern modules are equivalent to the motor command units in the optic lobe still remains.

The optic lobe is the largest brain area in cephalopods. It has been estimated that each of the paired optic lobes has a total of approximately 65×10^6 cells in *Octopus vulgaris* (Young, 1963). The periphery (cortex) of the optic lobe is a layered structure that contains visual processing units and is called “deep retina” (Young, 1971). In contrast, the center (medulla) of the optic lobe is a visual memory store as well as a higher motor center (Boycott, 1961; Young, 1971). It contains cell bodies of varying size, clumped together in characteristic “cell islands” surrounded by neuropil. Since there is no obvious histological patterning within the medulla, the neural circuitry and its functional organization have been difficult to examine.

Boycott (1961) was the first to apply direct electrical stimulation in the cephalopod brain and study the influence of chromatophore activation in the skin. Using hand-held electrodes to stimulate chromatophore lobes, lateral basal lobes, and optic lobes in lightly anaesthetized cuttlefish (*S. officinalis*), he was able to evoke skin coloration changes. Stimulating lower motor centers (anterior or posterior chromatophore lobes), cuttlefish showed total darkening on the head/arms or mantle ipsilaterally. Similarly, stimulating the lateral basal lobe (the upstream of the chromatophore lobe) led to uniform darkening, either ipsilaterally or bilaterally, never paling. The most interesting result came from stimulating the optic lobe, in which cuttlefish had one of three kinds of response: darkening, paling, or patterning, unilaterally or bilaterally. The patterns obtained included light mottle, zebra, and disruptive. In a subsequent study, by chronically implanting electrodes in the optic lobes of unrestrained cuttlefish and electrically stimulating this brain area, Chichery and Chanelet (1976) were able to elicit the deimatic pattern (used in intimidating or threatening behaviors) and other motor responses. These results support the hypothesis that the optic lobe is a motor command center, which controls a set of skin components for generating distinct body patterns. However, the neural organization of these specific motor command units in the optic lobe is still largely unknown.

Materials and Methods

Electrical stimulation

Following the lead of earlier investigators (Boycott, 1961; Chichery and Chanelet, 1976), we also electrically stimulated neurons in the optic lobe and video recorded the resultant body

pattern changes in cuttlefish *S. pharaonis* and oval squid *S. lessoniana*. To implant electrodes into the medulla of the optic lobe, animals were anesthetized using 3% and 2% MgCl₂ in cold sea water (15-18 °C) for cuttlefish and oval squids, respectively. Although urethane or ethanol has been commonly used as an anesthetic in cephalopods (e.g., Bullock, 1984), a recent study showed that magnesium chloride is better for long-duration anesthetization in cephalopods (Mooney et al., 2010). At least in the longfin squid *Doryteuthis pealeii*, MgCl₂ had no apparent effect on physiological evoked potentials recorded from nerve bundles within the statocyst system, which suggests the suitability of this solution as a sedating agent. To place the electrode in the optic lobe with a greater precision, we used a stereotaxic device (the one typically used in locating specific brain areas in rats/mice for precise measure) to assist the insertion of electrode and systematic mapping of these stimulation sites. This device also allowed us to implant electrodes at exact locations repeatedly in different animals. Although it is possible to insert the electrode directly through the skin and dorsal cranial cartilage into the optic lobe (e.g., Bullock and Budelmann, 1991), for ensuring the implantation site of the electrode, it is much easier to cut the cranium open for the cuttlefish preparation. We cut the dorsal skin of the head along an arc parallel to the wall of the orbit and expose the underlying tight connective tissue. This cut revealed the white body cushioning the optic lobe on all sides, and the electrode was implanted through the white body. In the oval squid preparation, the electrode was inserted directly through the skin and dorsal cranial cartilage into the optic lobe (e.g., Bullock and Budelmann, 1991). Stimulation was carried out by using an electronic stimulator (e.g., A-M Systems 2100), in which the output strength and frequency can be controlled accurately. By using a video camera to record the animal response upon electrical stimulation from above, we were able to systematically map the motor fields of command units in the optic lobe. To determine the exact site of electrical stimulation in the optic lobe, the imaging method (MRI or ultrasound) was applied and followed by serial sectioning via the cryostat.

Behavioral assay

Despite the success of using computer generated substrates (printed and laminated for water proof) in studying visual mechanisms of body patterning in cuttlefish, these static backgrounds do not permit us to examine the speed and transition of body patterning in response to visual input change. Even though we could suddenly change the background pattern by manually pulling away one substrate and revealing a different substrate underneath, we inevitably induced disturbance and animals were threatened. Under this circumstance, cuttlefish showed deimatic body patterns, rather than camouflage behaviors. It is ideal if one can use a computer controlled display as a substrate and dynamically change the background pattern without disturbing the animal in study, then the spatiotemporal expression of body patterns in response to visual input change can be assessed reliably. Previously, we have tried

using light-emitting displays as dynamically controlled substrates, such as CRT and LCD monitors. Unfortunately, cuttlefish did not settle down on these light-emitting backgrounds easily, and they did not show appropriate camouflage body patterns accordingly. A possible reason might be that light coming from below is unnatural (light naturally comes from above), and cuttlefish do not acclimate in these artificial environments. To overcome this technical issue, an alternative approach is to use the reflective display system, such as E-Ink technology (e.g., Amazon Kindle), as a dynamically modulated substrate to study the speed and transition of body patterning in response to visual input change. We put cuttlefish (*S. pharaonis*) on Kindle DX (9.7 inch diagonal E-Ink screen) with various background patterns (uniform, small checkerboard, and large checkerboard). This set of substrates was displayed on Kindle DX randomly at three different refresh rates (1 min, 10 sec, and 1 sec) to examine cuttlefish dynamic body patterning during slow, medium, and fast visual background changes, respectively. Cuttlefish skin coloration was imaged using a digital video camera to record the transition of body pattern change upon background changes.

Results

Stimulation at the same site with different voltages

(a)



(b)

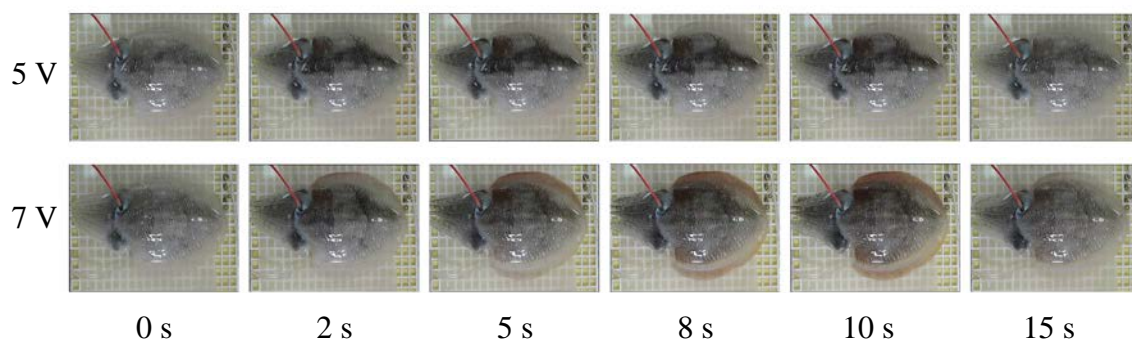


Figure 1 Responsive area of chromatophores is determined by given voltages of an impulse. (a) The anatomical image shows the stimulus location (arrow). (b) The time sequence of stimulation progress. 0 s means the beginning of the stimuli, and 10 s means the end of the stimuli. Five volts stimuli evoked darkening at ipsilateral head and arms, and displayed

anterior/posterior transverse mantle lines and white mantle bar patterns. Seven volts stimuli evoked general darkening head, arms, and fins with transverse mantle lines and white mantle bar patterns.

Stimulation at different depths of the medulla with the same voltage

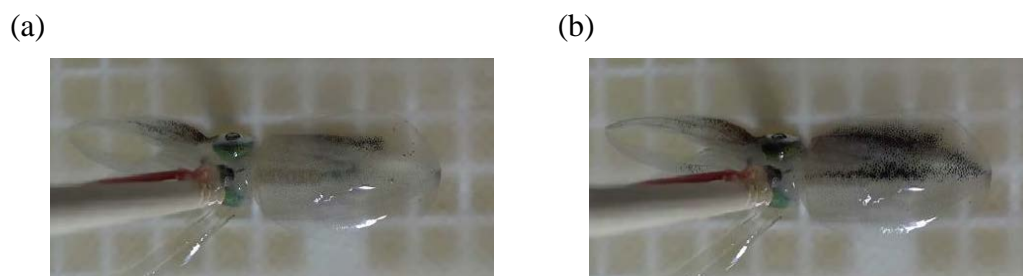


Figure 2 The responsive area is determined by the depth of the medulla. (a) Five volts stimuli evoked darkening at ipsilateral arms and fin edges. (b) Five volts stimuli at the depth which is deeper than (a) by 100 μm evoked darkening not only at ipsilateral arms and fin edges but also the mantle stripe.

Depth index and its relationship with the responsive area

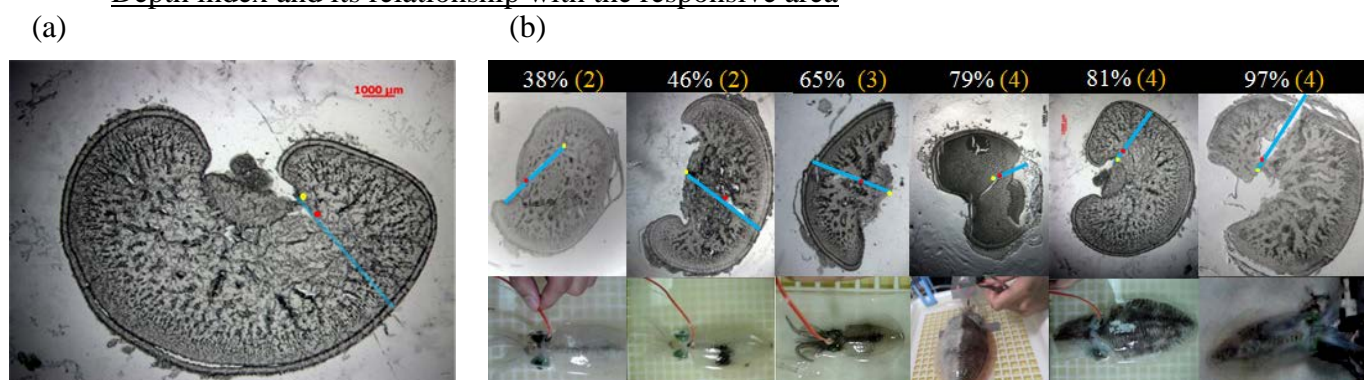


Figure 3 The responsive area is correlated with the depth index. (a) The depth index is defined that the distance from stimulating site (red dot) to cortical surface versus total length (yellow dot to cortical surface). The ratio can be defined into 4 zones. Zone 1: 0 to 25 %, zone 2: 26 to 50 %, zone 3: 51 to 75 %, and zone 4: 76 to 100 %. (b) Higher ratio of depth index is correlated with a larger responsive area.

Stimulation at different location of the medulla in cuttlefish



(b)

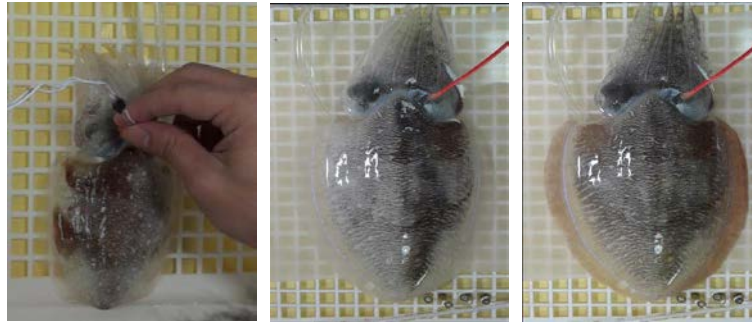
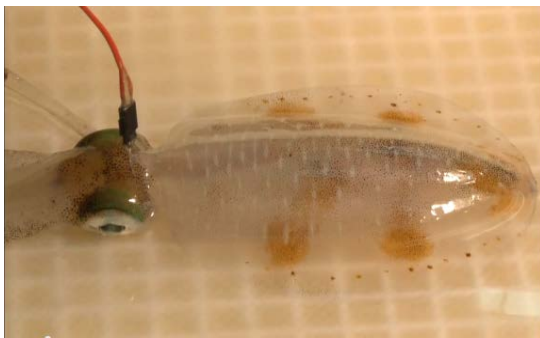


Figure 4 The pattern diagram induced by electrical stimulation in cuttlefish. (a) Dark arms and dark arm stripes. (b) White mantle bar, anterior mantle bar, posterior mantle bar, anterior and posterior transverse mantle lines.

Stimulation at different location of the medulla in oval squids

(a)



(b)



(c)



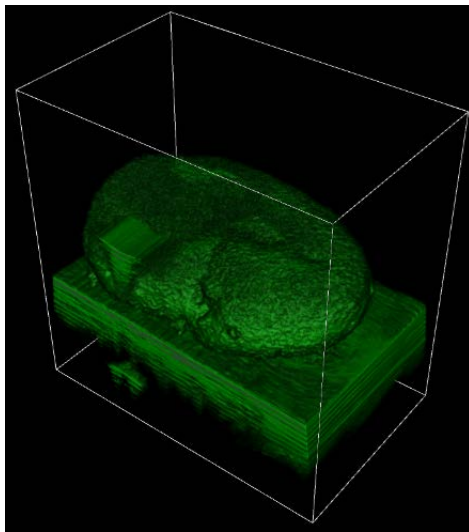
(d)



Figure 5 The pattern diagram induced by electrical stimulation in oval squid. (a) Fin margin spots and bands. (b) Mantle stripes and bands. (c) Dark uniform body. (d) Undefined patterns.

Acquisition of a precise map of neural organization underlying the body pattern control in the optic lobe

(a)



(b)

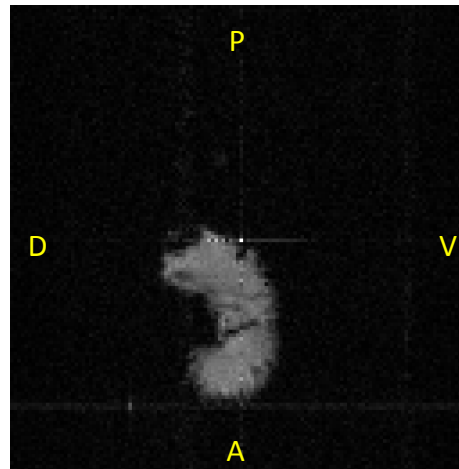


Figure 6 Visualization and characterization of the electrical stimulation sites in the optic lobe. (a) Ultrasound imaging is convenient to use and provides a reasonable spatial resolution. (b) MRI is slightly lower in spatial resolution, but the electrode localization data match well with those of the standard slice preparation.

Construction of the 3-D image of the standard optic lobe

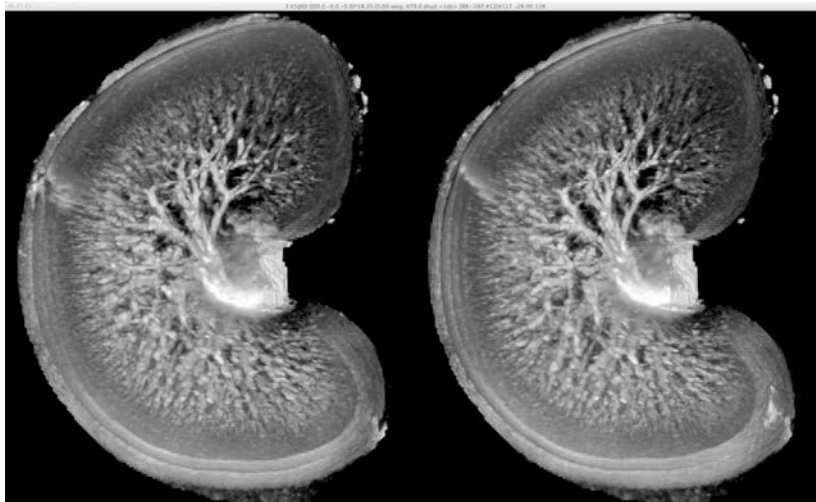


Figure 7 Stereo images of the optic lobe taken by Bruker 9.4 Tesla MRI (in collaboration with Prof. Samuel H.H. Chan and Dr. Chia-Hao Su at Kaohsiung Chang Gung Memorial Hospital, TAIWAN). The image rendition was made by Dr. Steve Senft (Marine Biological Laboratory, Woods Hole, USA).

Cuttlefish body patterning on the changeable display device

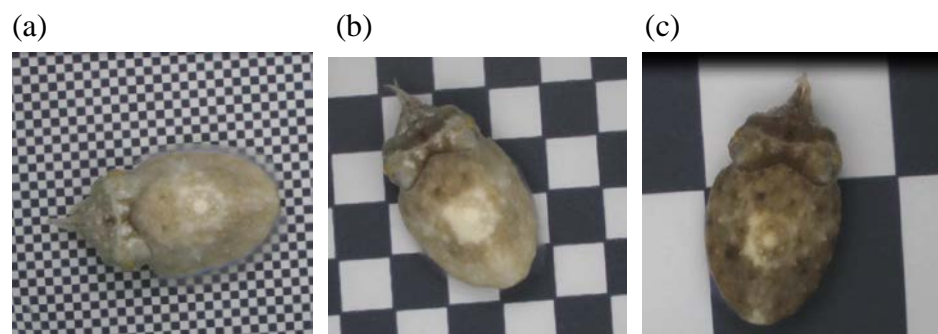


Figure 8 The same cuttlefish showed distinct body patterns on (a) small (b) medium, and (c) large checkerboard backgrounds displayed on the Kindle DX.

Table 1 Response time after switching the background of Kindle DX

	Latency of body pattern change	Latency of showing camouflage patterns
Day 1	18 sec	48 sec
Day 2	--	235 sec
Day 3	15 sec	74 sec

Note: The cuttlefish on Day 2 was nervous, thus the latency of showing camouflage body pattern was significantly delayed.

Modular organization of dynamic body patterning in cephalopods

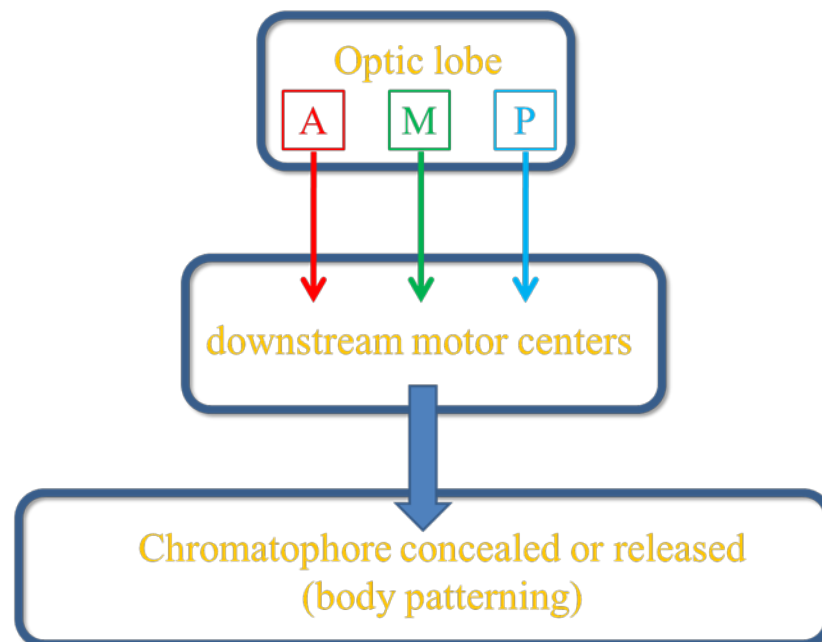


Figure 9 Motor command units in the optic lobe that independently control downstream motor centers for generating diverse body patterns

Presentation of this work in conference

Liu, T.-H. and Chiao C.-C. Neural organization of the optic lobe in controlling body patterns in of cephalopods. *2014 International Congress of Neuroethology*, Sapporo, Japan (July 28 – August 1). **Invited talk in a symposium**

Abstract

Cephalopods have the most sophisticated dynamic skin coloration for rapidly camouflage in nature. Previous studies have suggested that the pair of optic lobes located bilaterally in their brain plays a key role in controlling the expansion of chromatophores for generating diverse body patterns. However, the functional organization of the optic lobes and their neural control of various body patterns have not been examined systematically. In the present study, we applied electrical stimulation in the optic lobe to investigate the neural basis of body patterning in cuttlefish *Sepia pharaonis* and oval squids *Sepioteuthis lessoniana*. Animals were anesthetized by 3% magnesium chloride in sea water, and a tungsten electrode was inserted into their optic lobes with an aid of the stereotaxic instrument for electrical stimulation. The dynamic changes of body patterns were recorded by a video camera from above. We have observed that responsive areas of the skin upon electrical stimulation were positively correlated with increasing voltages and depths of the electrode in the medulla of the optic lobe. In addition, histological and brain imaging studies revealed that neurons aggregated as island-like clumps were varied systematically in the medulla, which suggests a hierarchical organization responsible for generating different body patterns.

References

- Anderson JC, Baddeley RJ, Osorio D, Shashar N, Tyler CW, Ramachandran VS, Crook AC, Hanlon RT (2003) Modular organization of adaptive colouration in flounder and cuttlefish revealed by independent component analysis. *Network* 14:321-333.
- Barbosa A, Mäthger LM, Buresch KC, Kelly J, Chubb C, Chiao C-C, Hanlon RT (2008) Cuttlefish camouflage: The effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vision Res* 48:1242-1253.
- Barbosa A, Mäthger LM, Chubb C, Florio C, Chiao C-C, Hanlon RT (2007) Disruptive coloration in cuttlefish: a visual perception mechanism that regulates ontogenetic adjustment of skin patterning. *J Exp Biol* 210:1139-1147.
- Boycott BB (1961) The functional organization of the brain of the cuttlefish *Sepia officinalis*. *Proc R Soc Lond B* 153:503-534.
- Bullock TH (1984) Ongoing compound field potentials from octopus brain are labile and vertebrate-like. *Electroencephalogr Clin Neurophysiol* 57:473-483.
- Bullock TH, Budelmann BU (1991) Sensory evoked potentials in unanesthetized unrestrained cuttlefish: a new preparation for brain physiology in cephalopods. *J Comp Physiol A* 168:141-150.
- Chan Y-C, Chiao C-C (2008) Effect of visual experience on the maturation of ON-OFF direction selective ganglion cells in the rabbit retina. *Vision Res* 48:2466-2475.
- Chen Y-C, Chiao C-C (2008) Symmetric synaptic patterns between starburst amacrine cells and direction selective ganglion cells in the rabbit retina. *J Comp Neurol* 508:175-183.
- Chiao C-C, Hanlon RT (2001a) Cuttlefish camouflage: visual perception of size, contrast and number of white squares on artificial checkerboard substrata initiates disruptive coloration. *J Exp Biol* 204:2119-2125.
- Chiao C-C, Hanlon RT (2001b) Cuttlefish cue visually on area - not shape or aspect ratio - of light objects in the substrate to produce disruptive body patterns for camouflage. *Biol Bull* 201:269-270.
- Chiao C-C, Kelman EJ, Hanlon RT (2005) Disruptive body patterning of cuttlefish (*Sepia officinalis*) requires visual information regarding edges and contrast of objects in natural substrate backgrounds. *Biol Bull* 208:7-11.
- Chiao C-C, Chubb C, Hanlon RT (2007) Interactive effects of size, contrast, intensity and configuration of background objects in evoking disruptive camouflage in cuttlefish. *Vision Res* 47:2223-2235.
- Chiao C-C, Chubb C, Buresch K, Siemann L, Hanlon RT (2009) The scaling effects of substrate texture on camouflage patterning in cuttlefish. *Vision Res* 49:1647-1656.
- Chiao C-C, Chubb C, Buresch KC, Barbosa A, Allen JJ, Mäthger LM, Hanlon RT (2010) Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. *J Exp Biol* 213:187-199.

- Chiao C-C, Wickiser JK, Allen JJ, Genter B, Hanlon RT (2011) Hyperspectral imaging of cuttlefish camouflage indicates good color match in the eyes of fish predators. *Proc Natl Acad Sci USA* 108:9148-9153.
- Chiao C-C, Hanlon RT (2013) Animal Camouflage. *McGraw-Hill 2013 Yearbook* (in press).
- Chichery R, Chanelet J (1976) Motor and behavioral responses obtained by stimulation with chronic electrodes of the optic lobe of *Sepia officinalis*. *Brain Res* 105:525-532.
- Dubas F, Hanlon RT, Ferguson GP, Pinsker HM (1986) Localization and stimulation of chromatophore motoneurons in the brain of the squid, *Lolliguncula brevis*. *J Exp Biol* 121:1-25.
- Hanlon RT, Messenger JB (1988) Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): The morphology and development of body patterns and their relation to behaviour. *Philos Trans R Soc Lond B* 320:437-487.
- Hanlon RT, Messenger JB (1996) *Cephalopod Behaviour*. Cambridge: Cambridge University Press.
- Hanlon R (2007) Cephalopod dynamic camouflage. *Curr Biol* 17:R400-404.
- Hanlon RT, Chiao C-C, Mäthger LM, Barbosa A, Buresch KC, Chubb C (2009) Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. *Philos Trans R Soc Lond B* 364:429-437.
- Hanlon RT, Chiao C-C, Mäthger LM, Buresch KC, Barbosa A, Allen JJ, Siemann L, Chubb C (2011) Rapid adaptive camouflage in cephalopods. In: *Animal camouflage: mechanisms and functions* (Stevens, M. and Merilaita, S., eds) Cambridge: Cambridge University Press.
- Kelman EJ, Baddeley RJ, Shohet AJ, Osorio D (2007) Perception of visual texture and the expression of disruptive camouflage by the cuttlefish, *Sepia officinalis*. *Proc Biol Sci* 274:1369-1375.
- Kelman EJ, Osorio D, Baddeley RJ (2008) A review of cuttlefish camouflage and object recognition and evidence for depth perception. *J Exp Biol* 211:1757-1763.
- Marshall NJ, Messenger JB (1996) Colour-blind camouflage. *Nature* 382:408-409.
- Mäthger LM, Barbosa A, Miner S, Hanlon RT (2006) Color blindness and contrast perception in cuttlefish (*Sepia officinalis*) determined by a visual sensorimotor assay. *Vision Res* 46:1746-1753.
- Mäthger LM, Denton EJ, Marshall NJ, Hanlon RT (2009) Mechanisms and behavioural functions of structural coloration in cephalopods. *J R Soc Interface* 6:S149-163.
- Messenger JB (2001) Cephalopod chromatophores: Neurobiology and natural history. *Biol Rev* 76:473-528.
- Mooney TA, Lee W-J, Hanlon RT (2010) Long-duration anesthetization of squid (*Doryteuthis pealeii*). *Mar Freshw Behav Physiol* 43:297-303.
- Packard A (1982) Morphogenesis of chromatophore patterns in cephalopods: Are

- morphological and physiological 'units' the same? *Malacologia* 23:193-201.
- Packard A (1995) Organization of cephalopod chromatophore systems: A neuromuscular image-generator. In: *Cephalopod Neurobiology* (Abbott, N. J. et al., eds), pp 331-367 New York: Oxford University Press.
- Young JZ (1963) The numbers and sizes of nerve cells in *Octopus*. *Proc Zool Soc Lond* 140:229-254.
- Young JZ (1971) *The Anatomy of the Nervous System of Octopus vulgaris*. Oxford: Clarendon Press.
- Zylinski S, Osorio D, Shohet AJ (2009a) Edge detection and texture classification by cuttlefish. *J Vision* 9:1-10.
- Zylinski S, Osorio D, Shohet AJ (2009b) Perception of edges and visual texture in the camouflage of the common cuttlefish, *Sepia officinalis*. *Philos Trans R Soc Lond B* 364:439-448.